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Preparation of hydrocortisone nanosuspension through a bottom-up nanoprecipitation technique using microfluidic reactors

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ABSTRACT

In this work, the possibility of bottom-up creation of a relatively stable aqueous hydrocortisone nanosuspension using microfluidic reactors was examined. The first part of the work involved a study of the parameters of the microfluidic precipitation process that affect the size of generated drug particles. These parameters included flow rates of drug solution and antisolvent, microfluidic channel diameters, microreactors inlet angles and drug concentrations. The experimental results revealed that hydrocortisone nano-sized dispersions in the range of 80–450 nm were obtained and the mean particle size could be changed by modifying the experimental parameters and design of microreactors. The second part of the work studied the possibility of preparing a hydrocortisone nanosuspension using microfluidic reactors. The nano-sized particles generated from a microreactor were rapidly introduced into an aqueous solution of stabilizers stirred at high speed with a propeller mixer. A tangential flow filtration system was then used to concentrate the prepared nanosuspension. The nanosuspension produced was then characterized using photon correlation spectroscopy (PCS), Zeta potential measurement, transmission electron microscopy (TEM), differential scanning calorimetry (DSC) and X-ray analysis. Results showed that a narrow sized nanosuspension composed of amorphous spherical particles with a mean particle size of 500 ± 64 nm, a polydispersity index of 0.21 ± 0.026 and a zeta potential of -18 ± 2.84 mV was obtained. Physical stability studies showed that the hydrocortisone nanosuspension remained homogeneous with slight increase in mean particle size and polydispersity index over a 3-month period.

1. Introduction

Recently, formulations containing nano-sized drug particles have been found to be promising candidates for enhancing the solubility of poorly water soluble drugs (Kesisoglou et al., 2007). A pharmaceutical nanosuspension is a submicron colloidal dispersion of drug particles which is stabilized by suitable stabilizers (surfactants and/or polymers) (Patravale et al., 2004). One of the main advantages of nanosuspensions is their small particle size and increased surface area which can lead to an increased dissolution rate and improved bioavailability (Rabinow, 2004). The applications of nanosuspensions in drug delivery (i.e. oral, parenteral, and pulmonary routes) have been reported (Chingunpituk, 2007). For ocular delivery, nanosuspensions of glucocorticoid drugs have been shown to enhance drug absorption rate and increase the duration of drug action (Kassem et al., 2007).

Techniques of drug nanosuspension preparation can be categorized into two principle classes; top-down and bottom-up technologies. The "top-down" technologies are the mechanical comminution processes of larger drug particles, as in milling (jet mills and pear-ball mills) and homogenization (high-pressure homogenizers). The "bottom-up" technologies begin with the molecules which are dissolved and then precipitated through non-solvent addition as in supercritical fluid (SCF) technology, spray-freezing into liquid process, evaporative precipitation into aqueous solution (EPAS) and liquid solvent change process (Li et al., 2007). Although "top-down" approaches are widely employed, the drawbacks associated with mechanical attritions processes, such as time consumption, intensive-energy use, introduction of impurities, inadequate control of particles size and electrostatic effects, promote greater interest toward "bottom-up" creation of nanoparticles (Dhumal et al., 2008; Hu et al., 2008).

As indicated by the name, the term microfluidics involves fluid in small scale channels (micro) devices (van der Woerd et al., 2003). Microfluidics can be defined as the science and engineering of systems in which fluid behavior differs from conventional flow theory primarily due to the small length scale of the system (Nguyen and Wereley, 2006). As demonstrated by the low Reynold's number in

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these microfluidic devices, liquid flow patterns are laminar. This means that streams of miscible fluids moving through microchannels flow in parallel, without turbulence and mixing will occur as a result of diffusion of molecules across the interface between fluids (Weibel and Whitesides, 2006).

The aim of this study is to investigate the possibility of producing a nanosuspension of hydrocortisone, a practically water insoluble glucocorticoid drug (Kassem et al., 2007) by controlled precipitation using a microfluidic technique. The impact of experimental parameters for particle formation including solvent–antisolvent flow rate, internal diameters of the microreactors, drug concentrations and inlet angles was studied. Characterization and physical stability of the obtained nanosuspension were also carried out.

2. Experimental

2.1. Materials

Hydrocortisone, Hc (98%), hydroxypropylmethylcellulose, HPMC (3.5–5.6 cP, 2% in H_2O) and sodium lauryl sulphate, SLS, were purchased from Sigma–Aldrich, USA. Kollidon© 30 (PVP K30), was purchased from BASF Aktiengesellschaft (Ludwigshafen, Germany). Other materials used were of analytical reagent grade.

2.2. Solubility study

The equilibrium solubility of Hc in water, ethanol, and in different ethanol/water combinations was determined by the shake flask method. Excess amounts of hydrocortisone were added to 2 ml of solvent. Vials were sealed to avoid changes due to solvent evaporation. The mixtures were shaken for 24 h at room temperature ($25 \pm 2\,^{\circ}C$) in a shaker (100 rev./min). After that the samples were left for 3 h to achieve equilibrium, and then filtered. An aliquot from each vial was adequately diluted and assayed spectrophotometrically (V-530 UV–vis spectrophotometer, Jasco, Japan) at wavelength of 247 nm to evaluate the amounts of drug dissolved. Experiments were carried out in triplicate.

2.3. Production of hydrocortisone nano-sized particles

2.3.1. Microfluidic parameters

Streams of an ethanolic solution of Hc and water were pumped through a set of microreactors (Epigem Ltd., UK) with different channels diameters (0.1, 0.5 and 1 mm) and inlet angles (10, 25 and 50°, Fig. 1-A). The effects of the parameters affecting the production of drug particles were studied. These variables include drug concentrations, flow rates, channels diameters and inlet angles.

2.3.2. Preparation of the hydrocortisone nanosuspension

Drug solution (10 mg/ml) and aqueous phase were mixed in a microfluidic reactor (10° inlet angle and 0.5 ml internal diameter) at a flow rate 1:2.5 ml/min respectively. Directly after emerging from the microreactor, the resulting dispersion was introduced to 115 ml of aqueous solution of stabilizer (0.2%, w/v, PVP + 0.2%, w/v, HPMC + 0.05%, w/v, SLS) with continuous stirring at 2000 rpm with a propeller mixer (Fig. 1-A). The resultant suspension was sonicated for 5 min followed by slow stirring at room temperature under reduced pressure for 5 h in order to decrease the ethanol content to very low residual levels in the aqueous dispersion (Pignatello et al., 2006; Kumar et al., 2008).

2.4. Concentration of nanosuspension

The Hc nanosuspension was concentrated at 20 psi transmembrane pressure (TMP) on a Minimate® tangential flow filtration (TFF) system with an Omega® 1K membrane (PALL Scientific, USA). The concentrated mode was adopted, where the nanosuspension was circulated through the TFF system using a peristaltic pump back to a reservoir until a concentrated volume has been collected. A fresh aqueous solution of stabilizers was added to the nanosuspension reservoir to ensure replacement and reduction of ethanol content to a minimal value (Fig. 1-B).

2.5. Determination of the drug content

The Hc content in the nanosuspension was assayed spectrophotometrically at 247 nm for the drug content after solubilization in ethanol and dilution.

2.6. Particle size measurement

The particle size of the produced dispersed systems was analyzed by photon correlation spectroscopy, PCS (Zetasizer® NanoS, Malvern Instruments, UK) yielding the mean particle diameter of the suspension. All samples were measured in the formed suspension after particle preparation without further dilution. The

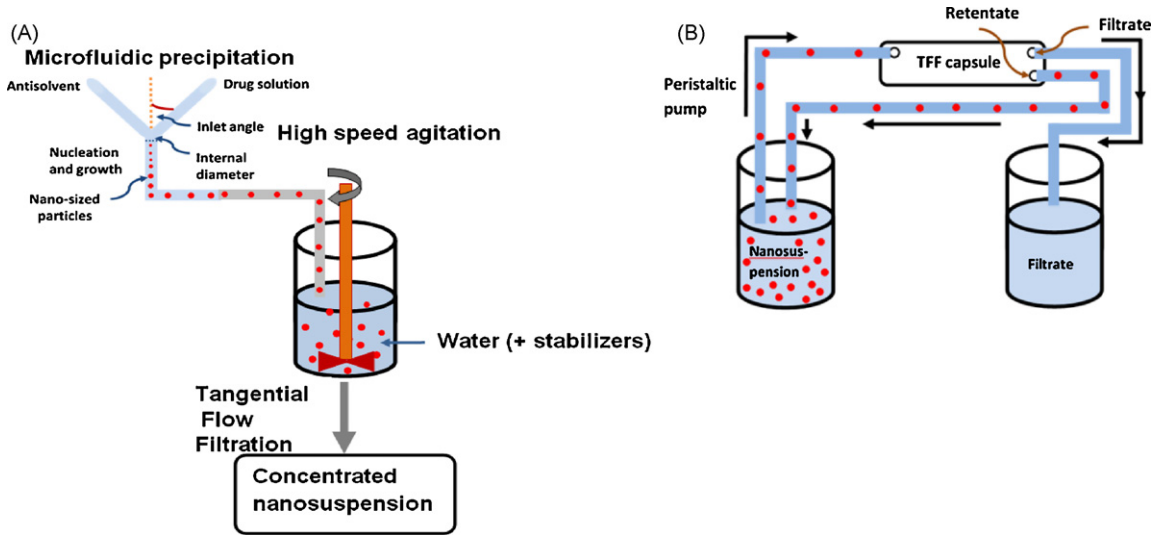

Fig. 1. (A) Diagram of nanosuspension preparation. (B) Schematic representation of tangential flow filtration (TFF) process.

measured parameters by PCS are the average particle size diameter (ZAve) and the polydispersity index (PI). The mean particle size of three measurements was taken.

2.7. Zeta potential measurement

Zeta potential of the nanosuspension was measured by a zeta meter (Zetasizer, Nanoseries, Malvern Instruments, UK). The measurement was performed after dispersion of the samples in water. The zeta potential (ζ) was calculated from electrophoretic mobility using Henry's equation (Agnihotri and Vavia, 2009):

$$U_E = 2\varepsilon\,\zeta f(K\alpha)/3\eta$$

where U_E is the electrophoretic mobility, ε is the dielectric constant of the suspending medium, ζ is the viscosity of the medium, K is the Debye-Hückel parameter, and $f(K\alpha)$ is a correction factor that takes into account the thickness of the double layer and particle diameter (α). The K unit is a reciprocal length. $1/K$ is frequently described as the thickness of the electrical double layer. Mean and standard deviation of five determinations were reported.

2.8. Scanning electron microscopy (SEM)

Samples of unprocessed Hc were examined using scanning electron microscopy by the Quanta 400 SEM (FEI Company, Cambridge, UK) after being mounted onto a graphite layer on an aluminum cylinder under vacuum.

2.9. Transmission electron microscopy (TEM)

Characterization of the external morphology of Hc nano-sized particles in suspension was determined using transmission electron microscopy (TEM). A drop of Hc nanosuspension was adsorbed on the surface of copper grid and dried at ambient temperature. Owing to the poor conductivity of organic samples, negative staining with an aqueous 2% magnesium uranyl acetate solution was applied for 2 min before TEM measurements. The sample was transferred into a TEM (JEM-1200EX, Japan Electron Optics Laboratory Corporation, Japan) operated at 120 kV.

2.10. Differential scanning calorimetery (DSC) analysis

A differential scanning calorimeter (TA Q2000, USA) was used to obtain DSC thermal profiles of hydrocortisone. Samples of processed and unprocessed Hc (≈4 mg) were run at a scanning rate of 10 °C/min under nitrogen atmosphere. The temperature for the scan ranged from 20 to 250 °C. Processed hydrocortisone solid sample was gathered from nanosuspension by centrifugation using an Avanti ultracentrifuge (Beckmann, USA) at 4 °C and rotate speed of 25,000 rpm for 30 min. The upper clear liquid was removed and the remaining residue was dried in a heated vacuum desiccator at of 40 °C and −25 inch Hg.

2.11. X-ray powder diffraction (XRPD) measurements

X-ray powder diffraction patterns of the dried Hc samples were obtained using a Siemens D5000 diffractometer (Siemens, Germany), using Cu Kα radiation (λ = 1.5418 Å). Samples of processed and unprocessed Hc were scanned over an angular range of 2–50° 2θ, with a step size of 0.05° and a count time of 3 s per step. Samples were rotated at 30 rpm during analyses. The generator was set to 40 kV and 30 mA.

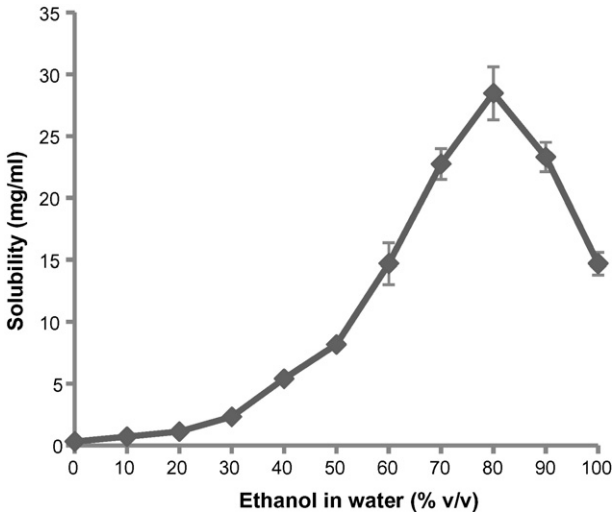

Fig. 2. Solubility of hydrocortisone in different ethanol–water combinations at 25 °C.

2.12. Physical stability study

Physical stability of the obtained Hc nanosuspension was investigated at 25 °C. The changes in appearance, particle size and polydispersity index were recorded over the period of 3 months. Settling behavior was monitored by visual examination.

3. Results and discussion

3.1. Solubility study

In order to obtain an indication of the amount of drug to be precipitated under experimental conditions, the solubility of Hc in different ethanol–water combinations were determined. The experimental ethanol/water solubility profile of Hc at 25 ± 2 °C is shown in Fig. 2. Solubility of Hc in water was found to be 0.311 mg/ml and it increased on addition of ethanol up to a maximum of 28.4 mg/ml at 80% ethanol/water and then decreased to a value 14.7 mg/ml in absolute ethanol. The observed maximum in solubility profiles in aqueous mixed solvents has been reported with other solutes (Ruckenstein and Shulgin, 2003). As seen from the solubility profile, combinations containing higher ratio of antisolvent (water) are favorable for the precipitation process.

3.2. Microfluidic parameters

As illustrated in Fig. 3, the precipitation process within the microreactor starts with supersaturation initiated by diffusion and

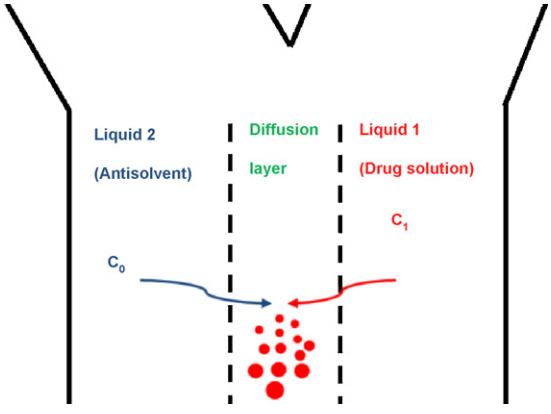

Fig. 3. Schematic representation of nanoprecipitation process within a microreactor.

Table 1
Effect of experimental parameters on mean particle size of hydrocortisone.

Flow rate				Channel diameter		Inlet angle	
Antisolvent: solvent (ml/min)	Mean particle size (nm)	Antisolvent: solvent (ml/min)	Mean particle size (nm)	Diameter (mm)	Mean particle size (nm)	Angle (°)	Mean particle size (nm)
0.25:0.25	445 ± 24	0.5:0.5	298 ± 15	0.1	N/A	10	230 ± 15
0.5:0.5	380 ± 19	1:0.5	271 ± 16	0.5	230 ± 15	25	275 ± 20
1:1	289 ± 31	1.5:0.5	207 ± 21	1	250 ± 18	50	298 ± 17
1.5:1.5	230 ± 15	2:0.5	152 ± 26				
		2.5:0.5	80 ± 19				

mixing between the solvent and antisolvent streams across the interface (diffusion layer) to drive nucleation and crystal growth. Solute depletion from diffusion layer as a result of nucleation and particle growth will be compensated by replenishments from drug solution stream. Micromixing time (τ_m) in "Y-shape" microreactors was found to be less than induction time of crystallisation (τ_i), which is essential to form nanoparticles with uniform size distribution (Zhao et al., 2007).

In a preliminary study, the impact of different processing parameters of the microfluidic precipitation process (flow rates of solvent and antisolvent, internal diameters of channels, inlet angles and drug concentration levels) on particle size was investigated at room temperature (25 °C). From these parameters, changes in flow rate were found to have the dominant effect on size of the generated particles. High flow rates (equal ratio) of drug solution and antisolvent resulted in smaller particles (Table 1). This behavior is attributed to the higher supersaturation level achieved at higher flow rates due to enhanced mixing (Su et al., 2007). The short residence time in microreactors will also decrease the tendency of particle growth. For fixed flow of alcoholic drug solution, increasing the flow rate of water, the antisolvent, resulted in a marked decrease in mean particle size (Table 1). Again, this was due to the higher initial supersaturation, due to the reduction of the solvent (ethanol) concentration. The increased antisolvent volume decreases the solute concentration on the formed particle surface. This was in accordance with Zhao et al. in preparing danazole nanoparticles using microchannel reactors (Zhao et al., 2007).

Results also generally indicated that as the internal diameters of the microreactor channels reduced, a slight decrease in particle size was observed. This could be attributed to higher mixing performance as a result of a higher share rate ($\overline{\gamma}$) in the outlet channel (collision zone) which is inversely proportional to the collision zone diameter according to the following equation (Aoki and Mae, 2006).

$$\overline{\gamma} = \frac{\overline{\mu}_c}{D_c}$$

where $\overline{\mu}_c$ is the mean velocity of each reactant fluid at the collision zone and D_c is the collision zone diameter.

Sharper inlet angles (10 and 25°) resulted in slightly reduced particle size (Table 1), which is thought to occur as sharper inlet angles allow the two inlet flows to meet and flow down the outlet without causing any disruption to either flow. Computational Fluid Dynamics (CFD) simulations studies showed that larger areas of no/low velocity (stagnant zones) appeared in higher inlet angles which account for larger particle formation as stagnant zones may allow particle growth (Brook, 2006). Results also indicated that size distribution of particles generated using the 50° inlet angle reactors exhibited a slightly broader distribution. This could result as the fluid flows meet on a more horizontal plane causing more disruption of the two flows as they flow into the outlet.

Other results (data not shown) revealed that slightly smaller sized particles resulted from higher drug concentrations, which may be attributed to higher supersaturation levels and nucleation rates obtained.

3.3. Preparation of hydrocortisone nanosuspension

In an order to prepare Hc nanosuspension, the Hc nano-sized particles (prepared using a 10°, 0.5 mm microreactor, drug concentration of 10 mg/ml and flow rate of 1:2.5 ml/min, solvent: antisolvent, respectively) were rapidly introduced to an aqueous solution of stabilizers stirred at 2000 rpm. The mean diameter of particles was found to increase from 260 ± 32 nm to 500 ± 64 nm upon the dilution process (Fig. 4-A). The polydispersity index (PI) is also an important parameter in nanosuspension technology, as it gives an indication about the width of particle size distribution as well as the long-term stability of nanosuspension. A PI value of 0.1–0.25 indicates a narrow size distribution whereas a PI value greater than 0.5 indicates a very broad distribution (Patravale et al., 2004). For the prepared hydrocortisone nanosuspension, a low PI was found to be 0.21 ± 0.026 indicating a good distribution profile.

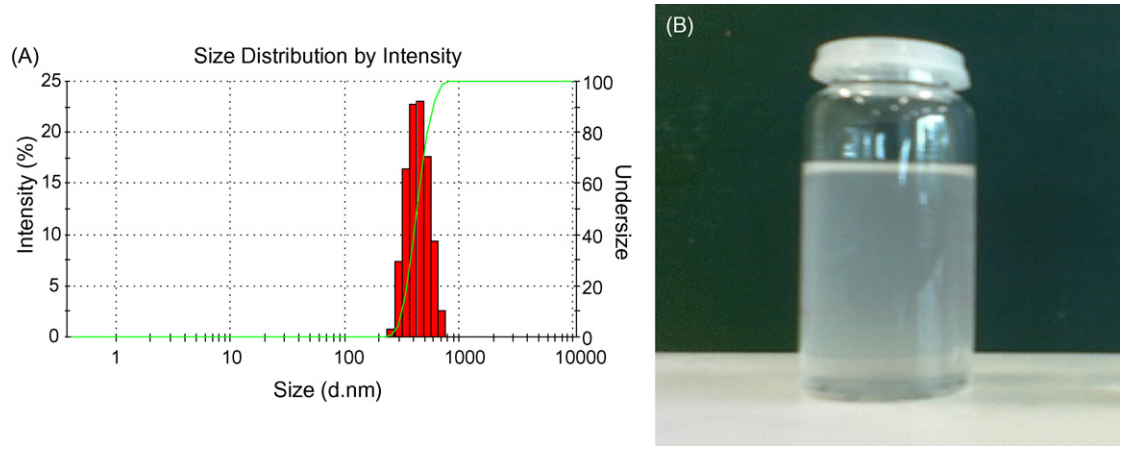

Fig. 4. (A) Particle size distribution of hydrocortisone nanosuspension. (B) Appearance of hydrocortisone nanosuspensions, 2 weeks after preparation.

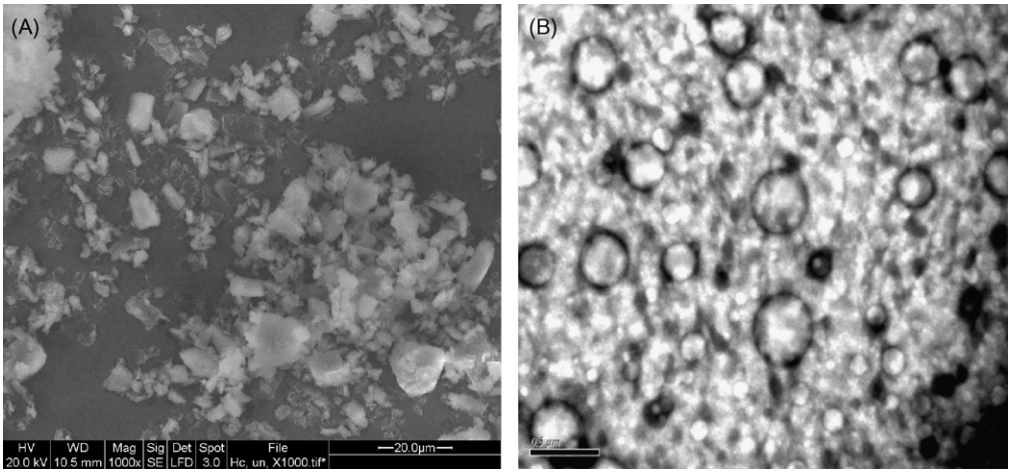

Fig. 5. (A) SEM micrograph of unprocessed hydrocortisone. (B) TEM micrograph nanosuspension.

A mixture of stabilizers (0.2% HPMC + 0.2% PVP + 0.05% SDS) was added to the aqueous phase in order to reduce the free energy of the system. The stabilizing effect by steric (coating of particles) and electrostatic (repulsion between particles) mechanisms provided by the polymers and ionic surfactants are thought to be complementary (see Fig. 4-B). PVP was added as a second polymer to enhance the stability of the nanosuspension. The combined effect of PVP and HPMC was also clear in stabilizing a carbamazepine colloidal system where HPMC–PVP interactions accumulated at the boundary layer around the drug particles thus leading to growth inhibition and additional prolonged stability (Douroumis and Fahr, 2007). Temperature changes can affect suspension systems stabilized by only polymer. Ionic surfactants improve stability against temperature changes for suspensions stabilized by polymers only and increase zeta potential values (Rabinow, 2004; Kesisoglou et al., 2007). In the absence of a significant energy barrier (stabilizers), the suspension containing only Hc showed rapid agglomeration immediately after preparation in order to reduce the free energy of the system.

3.4. Concentration of nanosuspension

After nanosuspesion formation, another crucial step for potential pharmaceutical application is to increase drug concentration. This process was carried out in this study using tangential flow filtration (TFF). In TFF, also known as cross flow filtration, the feed stream passes parallel, rather than perpendicular as in normal filtration, to the membrane face as one portion passes through the membrane (permeate) while the remainder (retentate) is recirculated back to the feed reservoir. The flow of sample solution across the membrane surface in TFF sweeps away aggregating molecules that form a "membrane-clogging" gel. Furthermore, TFF can be easily scaled up and conducted under aseptic conditions (Van Buitenen and Verrijk, 2003). The application of TFF is widely exploited in the area of biotechnology with expanding application in processing nano-sized pharmaceutical colloids (Dalwadi et al., 2005; Dalwadi and Sunderland, 2007). As seen from Table 2, the drug concentration was raised to 1 mg/ml using TFF while the mean particle size and PI were not affected.

3.5. SEM and TEM

Fig. 5 shows an SEM micrograph of the unprocessed Hc particles and a TEM image of the Hc nanosuspension. Unprocessed particles showed irregular shapes with particle size generally larger than 5 μm. Particles generated by nanoprecipitation using microreactors were primarily spherical with smooth surfaces and diameters slightly less than 500 nm, the diameter obtained by PCS. The minor difference in the measured size of the nano-sized particles between the two measuring techniques was expected because the sizes from PCS include any solvation shell and should therefore be slightly larger (Aboofazeli et al., 2000).

Table 2
Effect TFF on the drug concentration and particle size of hydrocortisone nanosuspension.

Formulation	Drug content (mg/ml)		Mean particle size (nm)		PI	
	Before	After	Before	After	Before	After
Hydrocortisone nanosuspension	0.65	1	500	500	0.21	0.21

3.6. DSC and X-ray analysis

The crystalline structure of Hc before and after nanoprecipitation was assessed by comparing the DSC thermal and XRPD profiles of unprocessed (raw Hc) and processed Hc (the powder recovered after centrifugation and drying of the nanosuspension). The DSC profiles showed a sharp peak at 224 °C, which corresponded to the melting point of Hc. In contrast, this peak disappeared completely in the DSC thermal profile of Hc particles from the nanosuspension (Fig. 6), suggesting an amorphous state of these particles. This

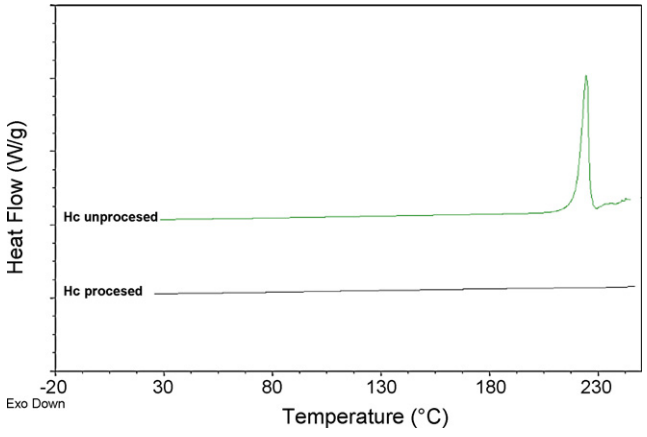

Fig. 6. DSC thermograms of unprocessed (raw) and processed hydrocortisone particles.

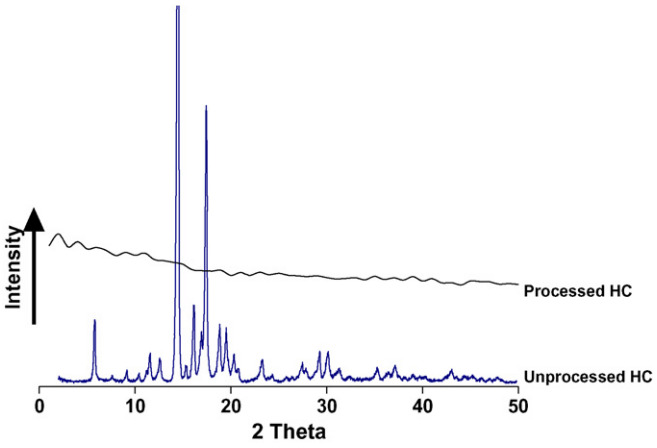

Fig. 7. XRPD of unprocessed (raw) and processed hydrocortisone particles.

finding was confirmed by comparing the XRPD spectra of the processed and unprocessed Hc (Fig. 7). The intense crystalline peaks at 2θ of 6, 14.5 and 17, observed in XRD spectrum of unprocessed Hc were absent in the XRPD spectrum of the dried Hc particles with a smooth line with no characteristic peaks. This evidence further supports the view that the crystalline structure had been lost as a result of the process of precipitation and drying.

3.7. Physical stability study

The storage stability of nanosuspensions is important, as finely dispersed particles in an aqueous phase have a high tendency to agglomerate together leading to the formation of larger aggregates. Ostwald ripening (the process of growing of larger particles on the expense of smaller particles due to enhanced solubility of the latter) presents an additional stability problem. Furthermore, due to the relatively high energy of the amorphous state, particles may relax to the lower-free energy crystalline form. The determination of the zeta potential (property related to the double electric layer on the surface of colloidal particles) of a nanosuspension is essential as it provides an indication about the physical stability of nanosuspensions (Patravale et al., 2004). A higher value of zeta potential indicates the likelihood of higher physical stability for systems stored under the same conditions. The measured zeta potential of the Hc nanosuspension was found to be $-18\,\text{mV} \pm 2.84$, a value within the range of ± 20 mV proposed for physically stable nanosuspensions stabilized by electrostatic and steric stabilization (Jacobs and Muller, 2002). After 3 months storage at room temperature, Hc nanosuspension remained homogeneous and no settling was observed. The mean particle size and PI increased to 687 nm and 0.235, respectively (Fig. 8). The amorphous status of Hc nanosuspension remained the same and no crystallinity was observed by DSC or XRPD analyses.

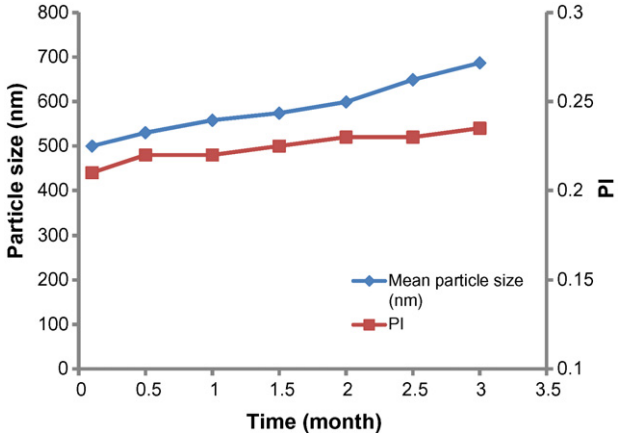

Fig. 8. Plot representing particle size and PI changes with storage time.

4. Conclusion

Microfluidics technique has been described as a simple method for drug nanosizing. The results have shown that a relatively stable aqueous hydrocortisone nanosuspension can be obtained using a bottom-up approach using microfluidic reactors. Particle size is directed by modifying the processing conditions and design of microfluidic reactors (internal diameters, and inlet angles). Changes in flow rates were found to have the dominant effect on size of the generated particles. The tangential flow filtration technique was efficient in concentrating the drug nanosuspension. These results illustrate the opportunity to formulate hydrocortisone in nanosuspension form as a drug delivery system for use in ophthalmic preparations. Further work on the ocular bioavailability of the prepared hydrocortisone nanosuspension is being carried out.

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